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Identification of Supernumerary der(20) Chromosomes by FISH in Three Patients

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Small supernumerary marker chromosomes of 3 patients were characterized at the molecular cytogenetic level. Two ring chromosomes and one metacentric marker were shown to be distamycinA/DAPI-negative and did not possess satellite regions after conventional banding techniques. Fluorescence in situ hybridization (FISH) was performed and in all 3 cases the supernumerary markers were shown to be derived from chromosome 20. Phenotypes are described and discussed with respect to karyotypes. Two of the patients are developmentally and/or phenotypically normal. The first patient has a ring chromosome, containing a small amount of euchromatic material; the second patient is the carrier of a small, metacentric and most probably heterochromatic marker. Patient 3 has physical anomalies, including a congenital heart defect and delayed motor development, but is intellectually almost normal. His marker chromosome is a ring containing a small amount of euchromatic material. Am. J. Med. Genet. 70:278–283, 1997.

KEY WORDS: supernumerary der(20) chromosomes; fluorescence in situ hybridization

INTRODUCTION

The identification of supernumerary marker chromosomes still remains a problem in cytogenetics, although the application of fluorescence in situ hybridization (FISH) using centromere and painting probes has facilitated the characterization of those markers [Crolla et al., 1992; Daniel et al., 1994; Højbjerg Gravholt et al., 1995]. Supernumerary marker chromosomes including extra ring chromosomes are estimated to occur with a frequency of 0.66/1,000 in newborn children [Nielsen and Wohlert, 1991] and in 0.4–1.5/1,000 prenatal diagnoses [Sachs et al., 1987; Blennow et al., 1994]. Their frequency is increasing to 3.27/1,000 in patients with mental retardation [Buckton et al., 1985]. Most marker chromosomes are derived from the short arms and pericentric regions of acrocentric chromosomes. Among these, inv dup (15) is the most frequent, constituting 57% of marker chromosomes in prenatal diagnoses [Blennow et al., 1994]. Several syndromes associated with supernumerary marker chromosomes are known. Mosaic i(12p) causes the Pallister-Killian syndrome [Larramendy et al., 1993; Van den Veyver et al., 1993]; an inv dup (22) is found in the "cat eye syndrome" [Liehr et al., 1992], and an i(18p) syndrome has also been described [Callen et al., 1990]. The occurence of additional der(20) chromosomes is rare and no common phenotype has been established. So far, there are 3 case reports of an extra r(20) ascertained postnatally [Blennow et al., 1993; Callen et al., 1991; van Langen et al., 1996] and one report with r(20) and a mosaic trisomy 20 cell line detected prenatally [Batista et al., 1995]. We describe 3 further patients with supernumerary marker chromosomes which are shown to be derived from chromosome 20 by FISH. These findings are compared to results of case reports published to date.

MATERIALS AND METHODS

Cytogenetics

Metaphase chromosome spreads were prepared from lymphocyte and amniocyte cultures according to standard methods. QFQ-, CBG-banding and DA/DAPI staining were performed by standard procedures.

FISH

FISH studies were performed using alpha satellite probes specific for chromosomes 2, 18, 20, X, Y (ONCOR, Gaithersburgh, MD) and library DNA probes specific for chromosome 20 (Angewandte Gentechnologie Systeme GmbH, Heidelberg, Germany). After hybridization according to manufacturer's instructions, probes were detected by incubation with avidin-fluoresceinisothiocyanate (Avidin-FITC). The amplification of sig-

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nals was performed by further incubation with biotinylated anti-avidin, followed by a second layer of avidin-FITC. Chromosomes were counterstained by DAPI (4′, 6-diamidino-2-phenyl-indole) and propidium iodide, dried and mounted in glycerol-based 1,4-diazabicyclo (2.2.2) octane antifading. The chromosomes were analysed with an epifluorescence microscope (Diaplan, Orthoplan, Leitz) and photographed with an Ektachrome 320 color slide film.

RESULTS

Patient 1

Cytogenetic analyses of an amniocyte culture were performed because of advanced maternal age. A monocentric supernumerary marker chromosome was detected in 80% of metaphases. In cord blood the marker was present at a frequency of 9% (karyotype: 46,XY/47,XY,+mar; QFQ). The parents' chromosomes were normal analysing lymphocytes from peripheral blood samples. Thus, the marker arose most probably de novo. Ultrasound analysis at gestational week 21 showed a normally developing fetus, although only one cord artery could be visualized by Doppler sonography. After genetic counselling, the pregnancy was continued to term. At the age of 20 months, the boy was developmentally and phenotypically normal except for an isolated syndactyly, type I of toes 2 and 3 (Table I, case 1).

Cytogenetics and FISH

Conventional cytogenetic investigations showed the marker to be a small ring chromosome since it appeared circular and was found in a mosaic. The marker was estimated to be smaller than 21q. It was pale after QFQ-banding (Fig. 1A), negative for DA/DAPI-banding, CBG-positive except for a small region and did not contain satellites. After comparing the marker chromosome with pericentric regions of QFQ-banded chromosomes, the following FISH probes were chosen: 2, 18, 20, and Y. Additionally we took advantage of sequence homologies of centromere-specific DNA belonging to the same alpha satellite DNA subfamily [Alexandrov et al., 1988]: for an efficient identification of derivatives from subfamily II, hybridization was performed with the centromere-specific alpha satellite probe D18Z1 under low stringency conditions. The probe gave bright signals on both chromosomes 18 and weaker cross-hybridization signals on the normal chromosomes 2, 20 and on the marker. Subsequently, hybridization with alpha satellite probe D20Z1 gave a bright positive signal for the marker (Fig. 2). Chromosome painting using the library probe specific for chromosome 20 was performed. Pericentric regions were slightly painted, whereas the centromere remained unstained (Fig. 3). This hybridization demonstrated the marker most probably to contain euchromatic material. Thus, FISH studies confirmed a chromosome 20 origin of the small ring chromosome [46,XY/47,XY, +mar.fish r(20) (D20Z1+, wcp20+)].

Patient 2

A monocentric marker chromosome was detected in 80% of metaphases in a fetus of a mother referred for amniocentesis because of advanced age (karyotype: 46,XX/47,XX,+mar, QFQ). Normal parental karyotypes of peripheral lymphocytes proved the marker to be most probably de novo. Ultrasound study at gestational week 22 showed a normally developing fetus. No morphologic abnormalities were observed. After genetic counselling, the parents decided to continue the pregnancy to term. At the age of 20 months, the girl was developmentally and phenotypically normal (Table I, case 2).

Cytogenetics and FISH

The marker was metacentric and approximately one-third the size of chromosome 21q. Q-banding gave no distinct banding pattern; the marker appeared pale (Fig. 1B). Furthermore, it was DA/DAPI-negative and CBG-positive. The presence of satellites was excluded. Subsequent FISH analyses were performed using repetitive centromere probes of chromosomes 2, 20 and X. These probes were chosen after comparing the marker chromosome with pericentric regions of QFQ-banded chromosomes. Hybridization with the alpha satellite probe D2Z gave bright signals on chromosomes 2 and weaker cross-hybridizations on both chromosomes 20 and the marker. To confirm these findings, hybridization with alpha satellite probe D20Z1 was performed and the marker was positive with this probe. Thus, the marker was shown to be derived from chromosome 20. Painting with library probe for chromosome 20 gave no signals for pericentric regions of the marker, showing that it contained no euchromatic material [46,XX/47,XX,+mar.fish der(20)(D20Z1+, wcp20-)].

Patient 3

The patient was referred to chromosome analysis at 2½ years because of a complex heart defect diagnosed as pulmonary atresia (Fallot type), cyanosis and clubbed fingers. His motor development was delayed, and he as not able to walk. His understanding was almost adequate for his age and he was able to speak 2–3 word sentences. Hypertelorism, low-set ears, depressed root of the nose and a bilateral moderate plantar furrow between the first and the second toe were noted. Height and weight were below the 3rd centile. After two unsuccessful heart operations, the boy was awaiting a heart-lung transplant. On examination at the age of 4 2/12 years, the transplant had not been performed. His height and weight were still below the 3rd centile. The patient was assessed as having global developmental delay with achievement at about the 25-month-old level with particular problems in gross motor development (30%) and social skills (40%). Speech development was almost in the normal range (70%); the fine motor area was less severely effected (50%).

In lymphocyte metaphases, 3 different cell lines were

TABLE I. Cytogenetic Findings and Clinical Data in 7 Patients With a Supernumerary der(20)

Case	Age	Karyotype	Cell system mosaic findings in %	Clinical findings	Reference
1	20 months	46,XY/47,XY,+r(20) de novo	Amniocytes (20:80%) Cord blood (91:9%)	Normal psychomotor development	Present investigation
2	20 months	46,XX/47,XX,+ der(20), de novo	Amniocytes (20:80%)	Normal psychomotor development	Present investigation
3	4 2/12 years	46,XY/47,XY,+r(20)/ 48,XY,+2 r(20), de novo	Lymphocytes (25:71:4%)	Global delayed development in gross motor, fine motor, speech and social skills, height and weight below 3rd centile, heart defect, hypertelorism, depressed root of nose, low-set ears, bilateral plantar furrow (1, 2), clubbed fingers	Present investigation
4	7 10/12 years	47,XY,+r(20)/48,XY, +2r(20), de novo	Lymphocytes (72:28%)	Normal intellectual development, height and weight below 3rd centile, high-pitched voice, scaphocephaly, low anterior hairline, synophrys, bushy eyebrows, flat philtrum, high palate, abnormalities of dentition, micrognathia, low-set abnormally folded ears, narrow shoulders, clinodactyly and partial cutaneous syndactyly, hyperextensible joints	Callen et al. [1991]
5	4 years	46,XY/47,XY,+r(20), de novo	Lymphocytes (52:48%)	Psychomotor retardation, behavioural abnormalities, slight growth retardation, pathologic hip mobility as a newborn, low-set ears	Blennow et al. [1993]
6	8 months	46,XY/47,XY,+r(20)	Lymphocytes (40:60%)	Psychomotor retardation, pronounced speech delay, occipital flattening, round face, deep-set eyes, upslanted palpebral fissures, strabismus, short nose, large nostrils, micrognathia, short neck, broad shoulders, clinodactyly, umbilical hernia, hypermobility, restricted mobility of hips	van Langen et al. [1996]
7	16 months	46,XY/47,XY, +r(20)/47,XY,+20/ 48,XY,+2r(20)	Amniocytes (10,5:44,7:44,7:0%) Chorionic villi (5,5:16,5:78,0:0%) Amnion (0:36,0:64,0:0%) Skin (16,7:80,6:1,6:1,1%) Cord blood (13,7:86,3:0:0%)	Delayed psychomotor development, height and weight below 3rd centile, hypotonia, asymmetric triangular face, prominent forehead, bulbous nose with slightly upturned tip, hypoplastic short philtrum, small mouth, high palate, micro- and retrognathia, abnormal ears, proximally placed adducted thumbs, clinodactyly, lymphedema on the dorsa of feet, abnormal position of toes, prominent heels, increased skin elasticity, hyperextensible joints	Batista et al. [1995]

found; 25% of the cells had a normal karyotype, while one extra marker chromosome was present in 71% and two extra markers in one metaphase, respectively (46,XY/47,XY,+mar/48,XY,+2mar,QFQ). After lymphocyte culture the parental karyotypes were normal. Thus, the marker arose most probably de novo (Table I, case 3).

Cytogenetics and FISH

The patient's marker was demonstrated to be a ring chromosome because of its circular appearance, mosaicism and the enlarged size in a few metaphases (partial duplication). The normal-sized marker had approximately half to one-third the size of a chromosome

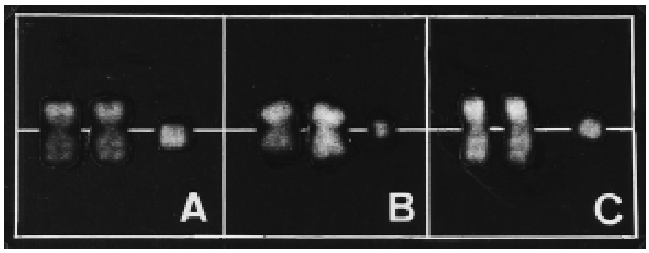

Fig. 1. QFQ-banded chromosomes. Demonstration of two normal homologues 20 and the supernumerary marker chromosome derived from chromosome 20. **A:** Case 1. **B:** Case 2. **C:** Case 3.

21q, showed no specific banding pattern and appeared mostly pale after QFQ-banding (Fig. 1C). It was negative for DA/DAPI and did not contain satellites. After comparison of pericentric regions of QFQ-banded chromosomes hybridization with alpha satellite probes of chromosomes 2, 20 and Y was performed. Signals on the marker chromosome were visualized with the centromere probe of chromosome 20 (D20Z1). One signal was seen on the normal-sized marker, and two signals on the enlarged marker chromosomes, demonstrating one and 2 centromeres, respectively. With the library probe specific for chromosome 20, the marker was slightly painted, proving that it most probably contained euchromatic material. Thus, FISH studies confirmed a chromosome 20 origin [46,XY/47,XY,+mar.fish r(20)(D20Z1+, wcp20+)/48,XY,+2mar.fish 2r(20)(D20Z1+, wcp20+)].

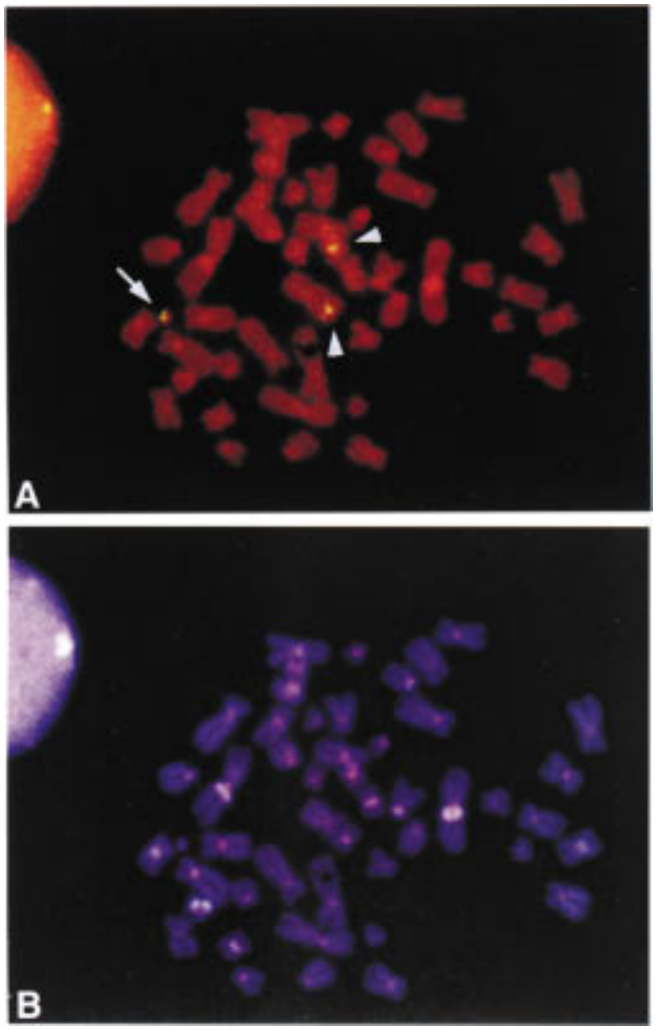

Fig. 2. **A:** FISH with the repetitive centromere probe D20Z1 to a metaphase spread of the patient in case 1. The marker chromosome is indicated by an arrow, the centromeric regions of the normal homologues 20 by arrowheads. Chromosomes were counterstained by propidium iodide. **B:** Counterstaining of metaphase by DAPI.

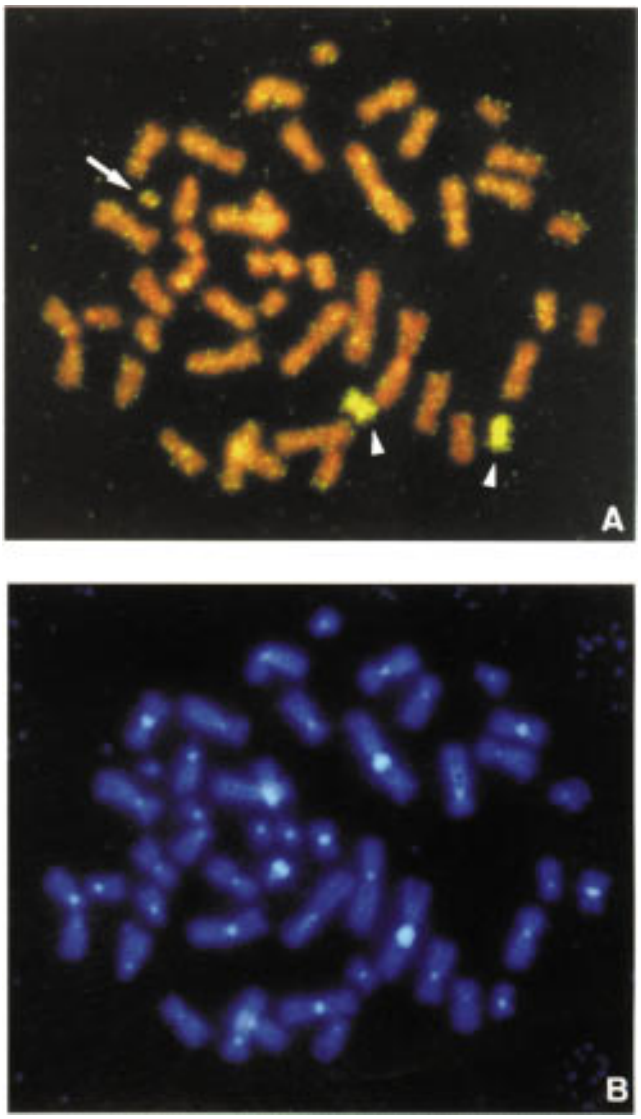

Fig. 3. **A:** FISH with the painting probe for chromosome 20 to a metaphase spread of the patient in case 1. The marker chromosome is slightly painted, whereas the centromere remains unstained. The marker is indicated by an arrow and the two normal homologues 20 by arrowheads. Chromosomes were counterstained by propidium iodide: **B:** Counterstaining of metaphase by DAPI.

DISCUSSION

The complete characterization of supernumerary marker chromosomes is needed for prognostic state-

ments, especially if de novo marker chromosomes are observed in prenatal diagnosis since there might be a risk of mental retardation and/or various abnormalities for the fetus.

Most marker chromosomes originate from chromosome 15 [Blennow et al., 1994]. These results are based on conventional cytogenetic investigations, e.g., DA/DAPI staining. Nearly 50% of all markers are DA/DAPI-positive and thus generally assumed to be derivatives of chromosome 15. Der(15) chromosomes are usually well characterized and much is known about the clinical outcome of the carriers, although the problem remains to distinguish inv dup(15) which give rise to phenotypic abnormalities from those which do not. The use of fluorescence in situ hybridization applying repetitive centromeric, library and locus-specific probes has greatly increased the possibility to assign markers to exact chromosome regions [Blennow et al., 1995; Brøndum-Nielsen et al., 1995; Cheng et al., 1994; Daniel et al., 1994; Højbjerg et al., 1995]. With this method, it was also possible to analyze marker chromosomes which occur less frequently, e.g., derivatives of 1, 4, 7, 8, 9, 20 [Blennow et al., 1993; Lanphear et al., 1995]. In contrast to well-known marker chromosomes such as i(12p), inv dup(15) or inv dup(22), there is still a general lack of information for these less frequent marker chromosomes.

We were able to analyze and characterize three supernumerary der(20) chromosomes. To our knowledge, only 3 reports of r(20) have been published as de novo mosaics at postnatal diagnosis [Blennow et al., 1993; Callen et al., 1991; van Langen et al., 1996; Table I, cases 4, 5, 6] and one case with a r(20) and an additional mosaic trisomy 20 cell line diagnosed prenatally has been described [Batista et al., 1995; Table I, case 7].

The patient described by Callen et al. [1991] had one supernumerary marker in 72% of the lymphocytes and 2 extra copies of the same marker in 28%. The 7 10/12-year-old boy was intellectually normal but with physical anomalies and growth retardation (Table I). The second patient [Blennow et al., 1993] was a 4-year-old boy who had a mosaic karyotype with the marker chromosome present in 48% of his lymphocytes. He showed psychomotor retardation and abnormal behavior, had slight growth retardation but no other physical anomalies. Van Langen et al. [1996] reported on a boy with an additional marker chromosome present in 60% of his lymphocytes with minor facial anomalies and developmental delay. Batista et al. [1995] reported on a 16-month-old boy who had a mosaic karyotype detected prenatally: 46,XY/47,XY,+r(20)/47,XY,+20. The boy showed delayed psychomotor development, physical anomalies and growth retardation. After birth, several tissues were investigated and the marker chromosome was found in all tissues at various frequencies (Table I). Since trisomy for chromosome 20 is one of the most common mosaic karyotypes detected in prenatal diagnosis and appears to be associated with a normal phenotype in liveborns and fetuses in 90% of cases [Hsu et al., 1991], the authors discussed the possible impact of the accessory marker chromosome on the patients' phenotype rather than an association with mosaic trisomy 20.

In contrast to these findings, we describe 2 patients with an additional der(20) who show no phenotypic defects or developmental retardation. Our patients have been followed to the age of 20 months. Since one has to take into account that the children may still develop retardation in the course of years, further follow-up of the patients will be necessary. Only one of 3 patients of the present study showed physical anomalies, growth retardation, delayed motor, speech and social development, and a congenital heart defect. According to these findings, we assume a relationship of the phenotype of the child with the additional der(20), especially since in this case a small amount of euchromatin has been proven in the marker.

Comparing our affected patient (case 3) with the 4 cases from the literature (cases 4–7), the only common findings were growth retardation and low-set ears/ abnormal ears in cases 4, 5 and 7. Additionally, our affected patient showed delayed motor development as it was described in cases 5, 6 and 7 published by other authors. Caused by the great variability of symptoms, there seems to be no typical syndrome associated with an extra der(20) chromosome. Phenotypic differences may be explained by following facts:

1. Variations in genetic content. The critical question is whether or not a given marker chromosome contains euchromatic material. Additional euchromatin leads to partial trisomies and may affect the patient. Of the cases described here, patients 1 and 3 had marker chromosomes most probably containing euchromatin. Patient 1 was normal and patient 3 showed physical abnormalities.

2. Variation of the degree of mosaicism between patients and between different tissues in the same patient. Six of 7 markers were found in a mosaic state.

3. Possibility of uniparental disomy for chromosome 20. Uniparental disomy of chromosome 20 has not been excluded in those cases discussed here. To our knowledge, only one case of UPD 20 has been reported which was caused by a rea(20;20) [Spinner et al., 1994].

The identification of more cases with der(20) chromosomes is needed for future interpretation. Further data may allow useful phenotypic comparisons to be made and thus to provide a better basis for genetic counselling.

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